PCR

Materials and equipment

- Thermocycler
- Microtubes (0.2 ml)
- Micropipette tips
- Micropipettes

Reagents

- Template DNA (cassette plasmid extraction)
- Standard reaction buffer 5X
- dNTPs
- Forward primer (VF2)
- Reverse primer (VR)
- DNA polymerase
- Nuclease-free water

Methodology

1. The procedure is as follows:
2. Thaw the template DNA, Standard reaction buffer 5X, dNTPs and primers (FP, RP) on ice.
3. Add in a 0.2 ml tube (for a 25µL reaction):
4. Up to 25 µL nuclease-free water
5. 5µL Standard Reaction buffer
6. 0.5 µL of 10 mM dNTPs
7. 0.5 µL of 10 µM Forward Primer
8. 0.5 µL of 10 µM Reverse Primer
9. 1µL of Template DNA
10. 0.125 µL DNA Polymerase
11. Mix gently each tube (by pipetting up and down several times).
12. Place at thermoblock in a program, based on the polymerase and primers specifications:
13. Denaturation step: 94˚C, 5 minutes
14. Second Denaturation: 94˚C, 30 seconds
15. Annealing: 56˚C, for 30 seconds
16. Extension: 68˚C, 1 minute
17. Final extension:68˚C, 5 minutes
18. Hold: 4˚C, infinite time
19. Repeat for 30-35 cycles from b-d.
20. Store PCR products at -20˚C.